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OR15-5 Human Sex Determination at the Edge of Ambiguity: Impaired SRY Phosphorylation Attenuates Expression of the Male Program

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Abstract

A paradox is posed by metazoan gene-regulatory networks (GRNs) that are robust yet evolvable. Insight may be obtained through studies of bistable genetic circuits mediating developmental decisions. A model in organogenesis is provided by the sex-specific differentiation of the embryonic gonadal ridge to form a testis or ovary. Here, we investigated a Swyer mutation in human testis-determining factor SRY that impairs its phosphorylation in association with variable developmental outcomes: fertile male, intersex, or infertile female (46, XY pure gonadal dysgenesis). The mutation (R30I) abrogates serine phosphorylation within a putative target site for protein kinase A (PKA) N-terminal to the HMG box. Diverse processes can be regulated by protein phosphorylation, including DNA recognition by transcription factors (TFs). Phosphorylation of this site in human SRY (LRR*SSS*FLCT; italics) *in vitro* was previously shown to enhance specific DNA affinity. Biological consequences of the mutation were evaluated in SRY-responsive mammalian cell lines following transient transfection. The mutation attenuated in concert occupancy of a target enhancer (TESCO) and *SOX9* transcriptional activation. These perturbations were mitigated by acidic substitution (LRI*DDD*FL) whereas Ala substitutions (RR*AAA*FL or RI*AAA*FL) attenuated activity to an extent similar to R30I alone. No differences were observed in nuclear localization. Mutagenesis suggested that the central Ser is most efficiently phosphorylated in accord with PKA targeting rules. Replacement of the native site by an optimized “Kemptide” PKA site (LRRASLGCT) enhanced both SRY phosphorylation and *SOX9* transcriptional activation whereas a “swapped” protein-kinase C determinant (LRRSSFRRCT) blocked phosphorylation. Among SRY variants, extent of cellular phosphorylation mirrored relative *in vitro* efficiencies of synthetic SRY-derived peptides as PKA-specific substrates. Although several kinases are predicted *in silico* to target this tri-serine motif, cell-based studies implicate PKA as the relevant kinase *in vivo*. Our results provide evidence that primate Sry requires its phosphorylation for full gene-regulatory activity. A PKA site N-terminal to the SRY HMG box, unique to primates, exemplifies network “tinkering” through recruitment of a new regulatory linkage. Molecular characterization of the R30I inherited Swyer

mutation in SRY thus demonstrates that impaired TF phosphorylation can attenuate a human developmental switch at the edge of ambiguity.